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ORIGINAL ARTICLE

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Identity and typification of *Achroomyces effusus* (Pucciniomycotina, Basidiomycota)

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Abstract
The identity of *Achroomyces effusus* is re-established with the use of morphological and DNA methods, and a neotype is selected. The species is conspecific with *Colacogloea peniophorae*, the generic type of *Colacogloea*, and has a priority over it. A new combination, *C. effusa*, is proposed.

Keywords Comb. nov. · Microbotryomycetes · Mycoparasite · Taxonomy

Introduction

The Pucciniomycotina (rust fungi and allies) are a subphylum of Basidiomycota heavily dominated by parasites, mainly inhabiting plants and other fungi. Only a few species, mostly with crust-like or pustulate basidiocarps, occur on plant remnants and are therefore considered as saprotrophs. A majority of these saprotrophic taxa belong to the Atractiellomycetes (*Helicogloea* Pat. and related genera) (Bauer et al. 2006; Aime et al. 2018; Spirin et al. 2018; Malysheva et al. 2020) and two species were assigned to the genus *Achroomyces* Bonord. (= *Platygloea* J. Schröt.). The generic type of the latter, *A. disciformis* (Fr.) Donk, produces pustulate or cushion-shaped, erumpent basidiocarps on still corticated branches of deciduous trees (almost exclusively on *Tilia cordata*). This species has been shown to be a member of the class Pucciniomycetes based on nrLSU sequence data (Bauer et al. 2006). Another species in the same genus, *A. effusus* (J. Schröt.) Mig., is a crust-like fungus appearing on wood of deciduous trees late in the season (Cohn 1889; Bourdot and Galzin 1909). Bandoni (1956) and Pilát (1957) treated it as a wood-inhabiting member of the genus, and this viewpoint has never been questioned. Only Krieglsteiner (1999) suggested *A. effusus* may be conspecific with *Colacogloea peniophorae* (Bourdot & Galzin) Oberw. & Bandoni (Microbotryomycetes, Pucciniomycotina), an intrahymenial fungus parasitizing corticioid fungi from the genus *Peniophorella* P. Karst. (Hymenochaetales, Agaricomycetes). However, Krieglsteiner's idea was based on anatomical similarity of the two species and has not yet been verified with the use of DNA methods. In the present paper, we select a type for *A. effusus* and investigate phylogenetic relationships of this species.

Material and methods

Collections from herbaria H, C, GENT, PC were studied. Herbarium acronyms are given according to Thiers (2020). Microscopic routine and terminology follow Spirin et al. (2018). The following abbreviations are used in taxonomic section: L – mean basidiospore length, W – mean basidiospore width, Q' – L/W ratio, Q – mean L/W ratio, n – number of measurements per specimens measured.

DNA study. We performed PCR directly from small fragments of dried basidiocarps without prior DNA extraction using the Phire Plant Direct PCR Kit (Thermo Scientific, USA) according to the manufacturer's instructions.

The following primers were used for amplification and sequencing: ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for the nrITS1-5.8S-ITS2 region,

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EF1-983F and EF1-1567R (Rehner and Buckley 2005) for a part of the *TEF1* region, JS1 (Landvik 1996) and LR5 (Vilgalys and Hester 1990) for D1-D3 domains of nrLSU region. PCR products were purified applying the GeneJET Gel Extraction and DNA Cleanup Micro Kit (Thermo Scientific, USA). There were two bands for the nrITS region, which were successfully separated during electrophoresis and then sequenced as separate templates. Sequencing was performed with an ABI model 3500 Genetic Analyzer (Applied Biosystems, USA). Raw data were edited and assembled in MEGA X (Kumar et al. 2018). Molecular studies were mostly carried out at the Center for collective use of scientific equipment "Cellular and molecular technology of studying plants and fungi" (Komarov Botanical Institute, Russian Academy of Sciences, St. Petersburg, Russia).

Phylogenetic analyses. For this study, five nrITS, one *TEF1* and three nrLSU sequences were generated. Additionally, 34 nrITS and 36 nrLSU sequences were retrieved from GenBank (www.ncbi.nlm.nih.gov/genbank/). Sequences were aligned with the MAFFT version 7 web tool (http://mafft.cbrc.jp/alignment/server/) with subsequent manual processing.

nrITS + nrLSU dataset comprised sequences from 15 genera accepted in Microbotryomycetes. The final alignment contained 1313 characters (including gaps). Phylogenetic reconstructions were performed with Maximum likelihood (ML) and Bayesian (BI) analyses. Before analyses, the best-fit substitution model for the alignment was estimated based on the Akaike Information Criterion (AIC) using FindModel web server (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). The "GTR plus Gamma" model was chosen for the nrITS + nrLSU dataset. ML analysis was run on RAxML servers, v.0.9.0 (Kozlov et al. 2019) with one thousand rapid bootstrap replicates. BI analyses were performed with MrBayes 3.2.5 software (Ronquist et al. 2012), for two independent runs, each with 10 million generations, under described model and four chains with sampling every 100 generations. To check for convergence of MCMC analyses and to get estimates of the posterior distribution of parameter values Tracer v1.6 was used (Rambaut et al. 2014). We accepted the result where the ESS (Effective Sample Size) was above 5700 and the PSRF (Potential Scale Reduction Factor) was 1.

Newly generated sequences have been deposited in GenBank with corresponding accession numbers (see below in the text). Sequenced specimens are marked by asterisk.

Results

Platygloea effusa J. Schröt. was described from Silesia (that time Germany, now Poland) as growing on rotten stumps (Cohn 1889). No original material of this species survived up to the present moment. Later it was discovered in some other European countries although considered rare everywhere (Bourdot and Galzin 1909; Wakefield and Pearson 1917; Bandoni 1956; Pilát 1957). Donk (1966) argued that *Achroomyces* has a priority over *Platygloea* J. Schröt., and therefore the species was treated as *A. effusus* since then (Wojewoda 1981; Hansen and Knudsen 1997; Krieglsteiner 1999). Fortunately, the protologue as well as the subsequent reintroduction of *A. effusus* by Bourdot and Galzin (1909, 1927) are explicit enough to allow recognition of the species, describing a crust-like fungus with four-celled, transversally septate basidia and hyaline, ellipsoid basidiospores. Two recent specimens of *A. effusus* were selected for morphological and DNA-based study.

The nrITS, nrLSU and *TEF1* sequences obtained from the two collections turned out identical to those ones of *Colacogloea peniophorae* in GenBank, thus confirming the aforementioned idea of Krieglsteiner (1999) about the identity of these two taxa. Additionally, two other ITS sequences generated from the same specimens ended up in *Peniophorella praetermissa* (P. Karst.) K.H. Larss. s. lato (GenBank MW293724, MW293725), the host species of *C. peniophorae*, although no signs of *P. praetermissa* basidiocarps (except a few hyphae and remains of cystidia) were detected in them. To verify these results, we studied and sequenced a typical specimen of *C. peniophorae*, which formed pustular, slimy fructifications (*Bruggeman 6343*) appeared directly on basidiocarps of *P. praetermissa*. ITS and LSU sequences of this specimen were found to be identical with those of *A. effusus* (Fig. 1). Since *A. effusus* has a priority over *C. peniophorae*, we combine it in the genus *Colacogloea* and treat the latter species as its synonym.

Colacogloea effusa **(J. Schröt.) V. Malysheva, Schoutteten & Spirin, comb. nov.**

MB838144

≡ *Platygloea effusa* J. Schröt. in Cohn, Kryptogamen-Flora von Schlesien 3 (1): 384, 1889. Neotype (here designated, MBT395027). Denmark. Midtjylland: Norddjurs, Løvenholm Skov, rotten wood, 26.VIII.2009 *Heilmann-Clausen 09–304** (C, isoneotype H) (GenBank ITS MW293723, LSU MW293727).

= *Platygloea peniophorae* Bourdot & Galzin, Bull. Soc. Mycol. France 25: 17, 1909.

Basidiocarps first hyaline, gelatinous, appearing as hardly visible patches or pustules on hymenial surface of *Peniophorella praetermissa*, then fusing together and finally forming perceptible (up to 5 cm wide and 1–2 mm thick), opalescent or yellowish, crustaceous fructifications with tuberculate hymenial surface, turning reddish or brownish in herbarium specimens. Monomitic; hyphae hyaline, clamped, thin-walled, mostly ascending, 1.5–2.5 µm in diam. Cystidia absent; hyphal endings abundant in

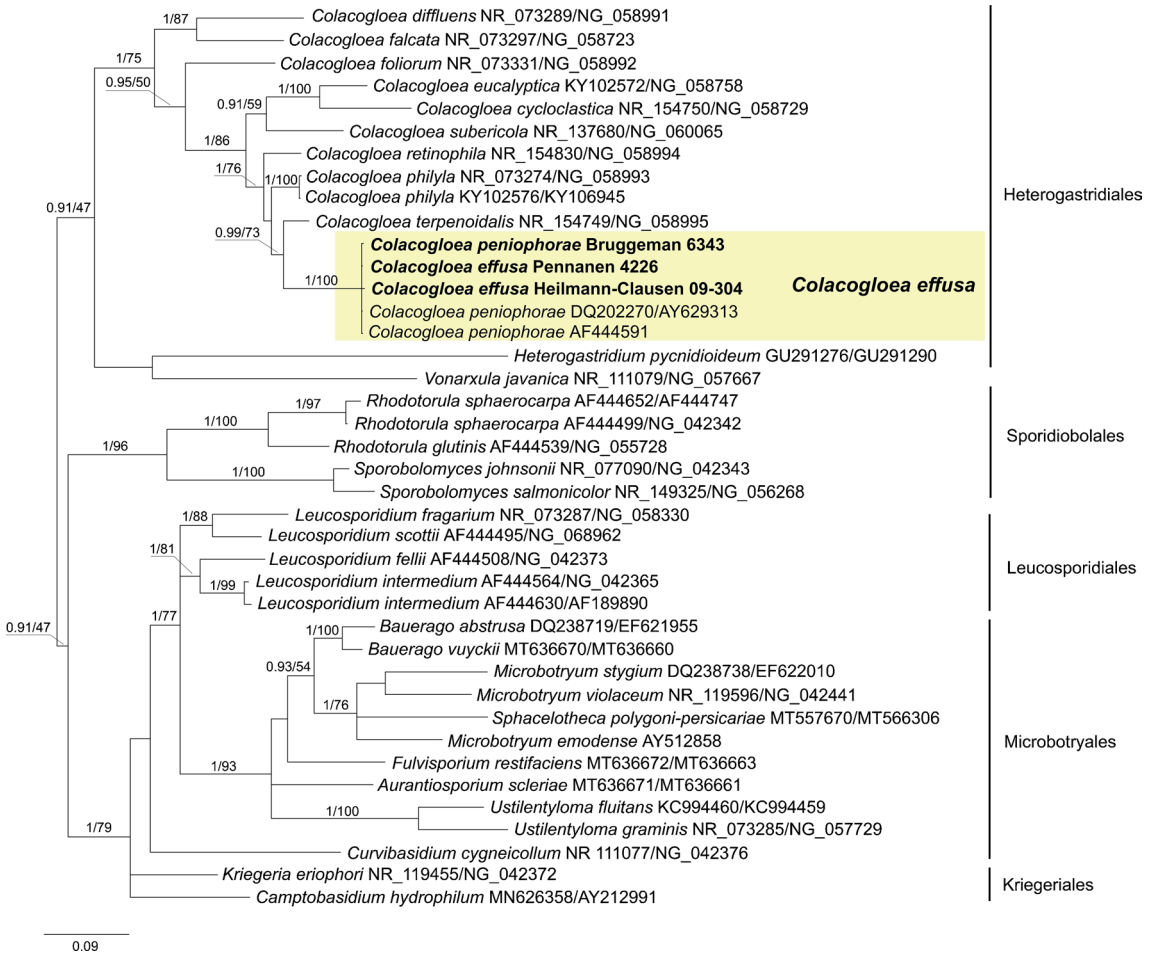

Fig. 1 Combined phylogenetic midpoint-rooted nrITS + nrLSU topology from Bayesian analysis showing main lineages within the Microbotryomycetes. All sequences generated for this study are indicated in bold and provided with collection numbers. GenBank accession numbers are given for all other sequences. Support values (BS/PP) are given above the branches. Scale bar shows expected changes per site

hymenium, simple or sparsely branched, 1–2 µm in diam. Basidia four-celled, transversally septate, narrowly clavate, usually somewhat winding or moderately curved at the apex, (44)48–80(83) × (4.0)4.1–5.3(5.8) µm (n = 30/2), probasidia absent; sterigmata up to 40 × 1.5–2 µm. Basidiospores hyaline, thin-walled, ellipsoid to bean-shaped, (6.2)6.8–9.2(–9.3) × (4.1)4.3–5.8(5.9) µm (n = 60/2), L = 7.83–8.03, W = 5.13–5.18, Q' = (1.3)1.4–1.8(1.9), Q = 1.53–1.56, with guttulate contents. Conidia present in some collections, hyaline, thick-walled, narrowly ovoid to subfusiform, often asymmetric or somewhat angular, 6–8 × 3–4(4.5) µm, distinctly cyanophilous.

Specimens examined. Finland. Varsinaissuomi: Raasepori, Framnäs, unidentified deciduous tree, 21.XI.2019 *Pennanen 4226** (H, as *A. effusus*) (GenBank ITS MW293722, LSU MW293726, *TEF1* MW298152). Netherlands. Utrecht: Zeist, Beerschoten, living *Peniophorella praetermissa* on decayed wood, 10.X.2019 *Bruggeman 6343** (GENT) (GenBank ITS MW303962, LSU MW310243).

Discussion

While introducing *P. peniophorae*, Bourdot and Galzin (1909) explicitly compared it with *Platygloea effusa* J. Schröt. (= *A. effusus*). They differentiated *P. peniophorae* from the latter species based on thinner and smaller basidiocarps, shorter basidia, presence of conidia, and its growth on corticioid fungi (*Peniophorella pubera* (Fr.) P. Karst. and *P. praetermissa* were listed as host species). According to our study, none of these features is reliable for separating these two taxa. In particular, the length of basidia is highly variable even in one specimen and seemingly correlates to the basidiocarp thickness. The presence of conidia is also unstable: of two collections of *A. effusus* mentioned above, one possesses abundant conidia (*Pennanen 4226*) while the neotype (*Heilmann-Clausen 09–304*) is completely devoid of them.

Of 23 authentic specimens of *P. peniophorae* in Bourdot's herbarium (PC), sixteen were collected from *P.*

praetermissa, one from *P. pubera* and six from unidentified corticioid fungi. Specimens from the first host species were evidently the main source for the Bourdot's original idea of *P. peniophorae*, and therefore this species should be treated as a synonym of *C. effusa*. Microscopic structures of *C. effusa* were illustrated by Bourdot and Galzin (1927), Wojewoda (1981) (as *A. effusus*) and Oberwinkler et al. (1990, as *C. peniophorae*). Macroscopic photographs of *C. effusa* can be found on Danmarks Svampeatlas web page (https://svampe.databasen.org, as *A. effusus*). Seemingly, *C. effusa* is able to consume its host species so aggressively that no visible signs of the latter are finally detectable. Certainly, this was a reason why *A. effusus* was believed to be a wood-inhabiting fungus. Nevertheless, a careful microscopic investigation and the use of molecular methods can re-confirm the presence of *P. praetermissa*.

Bandoni (1973) pointed out morphological differences of *C. effusa* specimens from different geographic areas. It may well be it represents a species complex. However, proving this hypothesis requires much more extensive sampling and incorporation of additional genetic markers. *Colacogloea effusa* is a generic type of *Colacogloea* Oberw. & Bandoni, a genus of which the majority of species is known only from asexual yeast stages (Wang et al. 2015; Li et al. 2020). Re-establishing its identity is important for future studies of species diversity and relations between sexual and asexual morphs in the Microbotryomycetes.

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Author contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by Vera Malysheva, Nathan Schoutteten and Viacheslav Spirin. Lab work and phylogenetic analyses were carried out by Vera Malysheva, Nathan Schoutteten and Annemieke Verbeken. The first draft of the manuscript was written by Viacheslav Spirin and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability DNA sequences used in the present study are available in GenBank. Alignments were deposited in TreeBase. Fungal specimens are stored in public herbaria (as indicated under Specimens examined).

Declarations

Ethics approval Not applicable.

Conflict of interests None.

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